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Charge transfer through a cytochrome multiheme chain: Theory and simulation

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ABSTRACT

We study sequential charge transfer within a chain of four heme cofactors located in the c-type cytochrome subunit of the photoreaction center of *Rhodopseudomonas viridis* from a theoretical perspective. Molecular dynamics simulations of the thermodynamic integration type are used to compute two key energies of Marcus' theory of charge transfer, the driving force ΔG and the reorganization energy λ. Due to the small exposure of the cofactors to the solvent and to charged amino acids, the outer sphere contribution to the reorganization energy almost vanishes. Interheme effective electronic couplings are estimated using ab initio wave functions and a well-parameterized semiempirical scheme for long-range interactions. From the resulting charge transfer rates, we conclude that at most the two heme molecules closest to the membrane participate in a fast recharging of the photoreaction center, whereas the remaining hemes are likely to have a different function, such as intermediate electron storage. Finally, we suggest means to verify or falsify this hypothesis.

1. Introduction

Charge transfer is a fundamental chemical reaction underlying important processes of life, such as photosynthesis, respiration or DNA damage and repair. Understanding these phenomena on a molecular level may also help to improve technical devices such as organic solar cells, sensors or functional, conducting nanostructures. Here, we focus on a model system of photosynthesis, the photoreaction center of the purple bacterium *Rps. virdis*.

In contrast to plants or cyanobacteria, purple bacteria conduct a strictly anoxygenic photosynthesis. By a series of cyclic electron transfer reactions, a reducing agent is recycled, and oxygen production is avoided. As a consequence, the photosynthetic apparatus remains comparatively simple and contains only a single photosystem, the so-called photosynthetic reaction center (PRC). Solving its structure [1] had been an outstanding contribution to understand photosynthesis, and the PRC remains an important model system to date. With the help of the structure presented in Fig. 1, we give a brief overview of the mechanism of harvesting light and converting its energy into a potential generated by the separation of two charges.

Upon irradiation, the so-called special pair of bacteriochlorophylls, P_{865} is excited with a main absorption maximum at the wave length of the index (in nm). The electron is rapidly transferred along the L branch of the protein to a bacteriophaeophytine (BPh b or bp) with the aid of an auxiliary bacteriochlorophyll b (BCl b). Subsequently, the electronic charge is transported along a pair of two quinones (Q_A, Q_B), probably assisted by the histidines coordinating an intervening iron ion [2]. Finally, the terminal molecule of the electron transfer chain, a ubiquinone (Q_B), will exit the PRC. To restart the photoreaction, the special pair cation has to be reduced by an electron localized on a charge carrier on the periplasmatic side of the protein.

In purple bacteria, the electron flow from the cytochrome bc_1 complex to the reaction center is usually mediated by the soluble electron carrier cytochrome c_2 [3]. In many species the photooxidized special pair P^+_{865} is not reduced directly by cytochrome c_2, but with the help of a multiheme cytochrome subunit, which is directly associated with the photoreaction center [4,5]. Although these multiheme cytochromes are known to act as an immediate electron donor to the special pair [6–9,22,23], the detailed mechanism of the electron transduction through the multiheme chain is still poorly understood. The structural organization of the four heme groups in the cytochrome subunit of the photoreaction center of *Rhodopseudomonas viridis* in an almost linear arrangement has become evident once the X-ray structure of the PRC was available [1], triggering the idea of a sequential downhill electron transfer process involving all four hemes. However, in its simplest version this view is not compatible with a strongly alternating midpoint potentials along the assumed charge transfer path [7,10–14,19]. Subsequent studies have shown that a fingerprint of four clearly distinguishable heme potentials can be found in many other photosynthetic bacteria [15–18]. Mutation experiments have demonstrated that charged amino acid residues in the vicinity of the heme groups have a crucial effect on their electrostatic properties and therefore make them controllable

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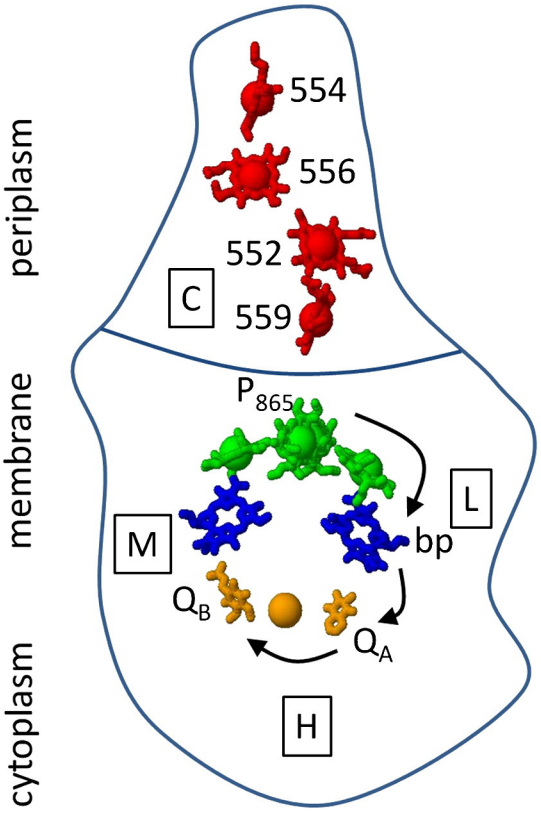

Fig. 1. Cartoon model of the heme electron transfer chain of the photosynthetic reaction center of *Rps. viridis* based on the structure of ref. [4].

to a certain extent [19,20]. Chen et al. have shown that the transfer rates of the interheme electron flow are particularly sensitive to changes in the redox potential of the hemes involved [21].

In the cytochrome subunit of the reaction center of *Rps. viridis* electrons are believed to be transferred through a linear chain of four heme groups to the special pair bacteriochlorophyll dimer P_{865}, the site of the primary photooxidation [7,11]. The midpoint potentials of the four heme groups have been determined experimentally, they are arranged in a low–high–low–high pattern starting at the periplasm side of the complex: heme-c_{554} ($E_M = -60$ mV), heme-c_{556} ($E_M =$ 320 mV), heme-c_{552} ($E_M = 20$ mV) and heme-c_{559} ($E_M = 380$ mV). Heme-c_{559} is the closest cofactor to the special pair and transfers an electron to P^+_{865} in 100–200 ns, depending on temperature and redox states of the heme groups [22–25]. It is well established that heme-c_{559} is the direct electron donor to the oxidized special pair [26]. The oxidized heme-c_{559} is then rereduced on a time scale of 2 μs by an electron transfer involving heme-c_{556} and heme-c_{552} [10,22,23,26]. To our knowledge, direct evidence for the functional role of heme-c_{556} is still missing. However, on the basis of kinetic studies it has been suggested that the first heme is the electron acceptor for the soluble electron donor cytochrome c_2 [27–29].

Despite extensive spectroscopic studies [10,11,23,25,30–32] the charge transfer processes within the cytochrome subunit of the photoreaction center of *Rps. viridis* have not been understood completely. Open questions include (i) the function of the four heme moieties, especially the role of the two low-potential hemes, (ii) the impact and biological function of the low–high–low–high arrangement of the heme midpoint potentials and (iii) the detailed pathway of the electron transfer through the subunit. From a theoretical perspective, Bombarda and Ullmann have addressed these questions using an electrostatic continuum model both for the protein and the solvent [33].

The remaining part of this article is organized as follows. In the following section, we will present the technical details of the molecular dynamics simulations and the associated thermodynamic integration scheme, leading to two parameters of Marcus' theory of charge transfer, the driving force and the reorganization energy. In the third section, we describe the electronic structure computations leading to the effective electronic couplings within the heme chain. In the fourth section, the results are integrated into Marcus' theory to compute charge transfer rates. The results are discussed, and conclusions are derived in the final section of the paper.

2. Molecular dynamics and thermodynamic integration

2.1. Force field parameters

While the standard force fields used in the *Amber* molecular modeling suite [34] are designed for the simulation of organic molecules and large biomolecules as proteins or nucleic acids, they are not able to describe transition metal complexes appropriately. Giammona [35] has generated force field parameters for the heme group that can be used supplementary to the *Amberff99SB* force field [36]. These parameters describe a heme group with a Fe^{2+}-ion as central atom. When simulating an interheme electron transfer reaction, a force field must also be able to describe the change of the oxidation state of the iron ion and the resulting change of the charge distribution in the ligand system.

In compounds containing late transition elements such as iron, correlation effects play an important role. They can usually not be adequately described by a Hartree–Fock electronic structure computation, which is the basis of the standard parametrization scheme of the *Amber* molecular modeling suite within the *Antechamber* routine. Hence, we took refuge to ab initio density functional theory for the computations of the missing force field parameters. Based on geometry optimizations using the OLYP functional and a 6-311G basis set, we have calculated the atomic partial charges for both the Fe^{2+} and the Fe^{3+} heme group. The resulting charge distribution is characterized by an excess charge that is not confined to the central iron atom, but is extended over a considerable fraction of the porphyrine system.

The thus computed excess atomic partial charges have been added to the *Amberff99SB* parameters and the resulting force field tested within a standard molecular dynamics simulation of the cytochrome subunit. In this simulation, a model system containing the protein backbone, all four heme cofactors and a 10 Å box of about 12,000 TIP3P water molecules has been used. The system has been subject to a 5000 steps steepest decent minimization followed by a 30 ps temperature equilibration up to 300 K in a *NVT* ensemble and a 40 ps *NPT* volume equilibration at 300 K. The molecular dynamics simulation was finally conducted in a *NPT* ensemble for 2 ns with snapshots of the protein geometry being taken each femtosecond. These structure snapshots served a geometrical basis for the electronic structure computations described in the next section.

2.2. Thermodynamic integration

Based on the preequilibrated structure of the cytochrome subunit of the bacterial photoreaction center several model systems with different charge distributions were generated, only the four heme molecules act as potential centers of charge localization. We use a variant of the thermodynamic integration (TI) scheme [37] adapted to charge transfer processes [38,39] and refer the reader to these papers for the technical details.

In a thermodynamic integration, an additional parameter Λ is introduced into the potential energy of the system; it acts as an interpolation parameter between the potential energy of an educt and a product state. For charge transfer processes, the only difference between these potential energies lies in their charge distributions. Integrating the derivatives of the potential energies with respect to

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